Anti-oxidant properties of different extract of *Pleurotus ostreatus.*

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Abstract

The different extracts of dried *Pleurotus ostreatus* mushroom namely, ethanol extract, methanol extract, ethyl acetate extract and chloroform extract were analyzed for antioxidant activity in different assays, namely, scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals and total phenolic content. Among the four mushroom extracts, the ethanolic extracts from *Pleurotus ostreatus* showed the most potent radical scavenging activity showing 86.33% followed by methanol extract 75.21%. Total phenolics content in the ethanolic extract of *Pleurotus* mushroom was highest in 248 µg equivalent of BHT/g followed by methanol extract 218 µg equivalent of BHT/g. Chloroform extract showed the least phenol content and antioxidant activity.

Keywords: Phenolic content; Antioxidant properties; *Pleurotus ostreatus*

Introduction

Mushroom is a well known fungus and is of great importance in modern era because of its use as diet supplement as well as its medicinal properties not only in India but all around the world. White Oyster mushroom is the most popularly grown variety of mushrooms in India both under seasonal and controlled environmental condition, for consumption in India and export. Oyster mushroom requires colder temperatures for its growth/fructification and that is why it is also referred to as temperate mushroom in literature. They are documented as being good source of nutrients and bioactive compounds that are beneficial to the human body (Chang, 2011). Documented literature indicates that mushrooms have photochemicals and other compounds which are strong antioxidants (Fang et al., 2002; Liu, 2004). Phenolic compounds, alkaloids, saponins, flavonoids, tannins, sterols, triterpenes, coumarins and cyanogenic glycosides have been detected in wild mushrooms. The antioxidant status in human reflects the vibrant balance between the antioxidant defense and prooxidant conditions and this has been suggested as a useful tool in estimating the risk of oxidative damage. ROS have been implicated in the pathophysiology of various clinical disorders, including ischemia, reperfusion injury, myocardial infarction, rheumatoid arthritis, neurodegenerative, atherosclerosis, acute hypertension, hemorrhagic shock and diabetes mellitus.

Fruiting bodies of *Pleurotus* possessed higher concentration of antioxidants than other commercial mushrooms. (Yang et al 2002) This activity was mainly due to presence of polysaccharide pleuran(S-glucan) that has been isolated from *P.ostreatus* showing a positive effect on rat colon with pre-cancerous lesions.(Bobek et al 2001) P. ostreatus increased the activities of important antioxidant enzymes (viz superoxide dismutase, catalse and peroxidase)thereby reducing oxidative damage in humans. Oyster mushrooms are now widely used as ingredients in dietary supplements in the hope of maintaining health and preventive diseases due to their higher free radical scavenging activities of oyster mushrooms depend upon the colour of fruiting bodies as per Yang et al. And these phenolic compounds have free radical scavenging property that reduces inhibitory effects of mutagens and carcinogens. Recently a very surprising result was shown by Saha et al. 2000 that juvenile bud stage(one day stage) contained highest amount of phenols (2.79mg/g) and

anti-oxidants that gradually decreases (1.27 mg/g) but upon maturity(four day stage), the total concentration of total phenol was again increased (2.08mg/g). Similar result was found earlier by Iwalokun et al, when they compare the in vitro antioxidant capacity of acetone extracts and petroleum ether extract. Recently Venkatkrishnan et al 2010 have shown that extract from *P.ostreatus* inhibited the growth of HL-60 cells by cell cycle arrest i.e by the induction of apoptosis by their experiments due to the presence of flavonoid (quercetin equivalent) and phenolics compound (cathechin equivalent) in fruiting bodies. Methanolic extract of *Pleurotus* significantly enhanced the activity of antioxidant enzymes.

Materials and Methods

Determination of Total Phenolics:

The amount of total phenolics in the extracts was determination by the modified Folin-ciocatleu method Wolfe et al. An aliquot of the extracts was mixed with 5ml F-ciocalteu reagent (previously diluted with 1:10 v/v) and 4ml (75g/1) of sodium carbonate. The tubes were vortexed for 15 sec and allowed to stand for 30 min at 40^0**C**for color development. Absorbance was then measured at 765nm using the UV- spectrophotometer. Samples from extract were evaluated at a final concentration of 0.1mg/ml. Total phenolics content was expressed as mg/g gallic acid equivalent.

Total Antioxidant Activity:

Total antioxidant activities of crude extracts were determined according to the method of Prieto et al. Briefly 0.3 ml of samples was mixed with 0.3 ml reagent solution (0.6m sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). Reaction mixture was incubated at 95 ^{0}C for 90 min under water bath. Absorbance of all the sample mixture was measured at 695nm. Total antioxidant activity is expressed as the number of equivalence of ascorbic acid.

DPPH Radical- Scavenging Activity: The scavenging effect of samples for DPPH radical was monitored according to the method of Yen and Chen. Briefly, a 2.0 ml of aliquot of test sample was added 2.0ml of 0.16mm DPPH methanolic solution. The mixture was vortexed for 1min and then left to stand at room temperature for 30min in the dark and its absorbance was read at 517nm. Synthetic antioxidant, Gallic acid and ascorbic acid were used as positive controls. The ability to scavenge the DPPH radical was calculated using the formula,

$$\textbf{Radical scavenging effect (\%)} = \frac{\textbf{Ab- As}}{\textbf{Ab}} \times \textbf{100}$$

Where, Ab = Absorbance of blank, As = Absorbance of Sample.

Result and Discussion

The oyster mushroom *Pleurotus* spp was purchased from Mushroom Centre, DRRPCAU. Pusa, Samastipur. This was extracted by using four different solvents like ethanol, methanol, chloroform and ethyl acetate and screened for its antioxidant activity.

Total Antioxidant Activity: Graph 1 indicated that the good antioxidant activity was observed at ethanolic extract of *P. ostreatus* (248 µg equivalent of BHT/g). Methanol also showed good antioxidant activity (218 µg equivalent of BHT/g) next to ethanol and chloroform exhibited least antioxidant activity (88 µg equivalent of BHT/g).

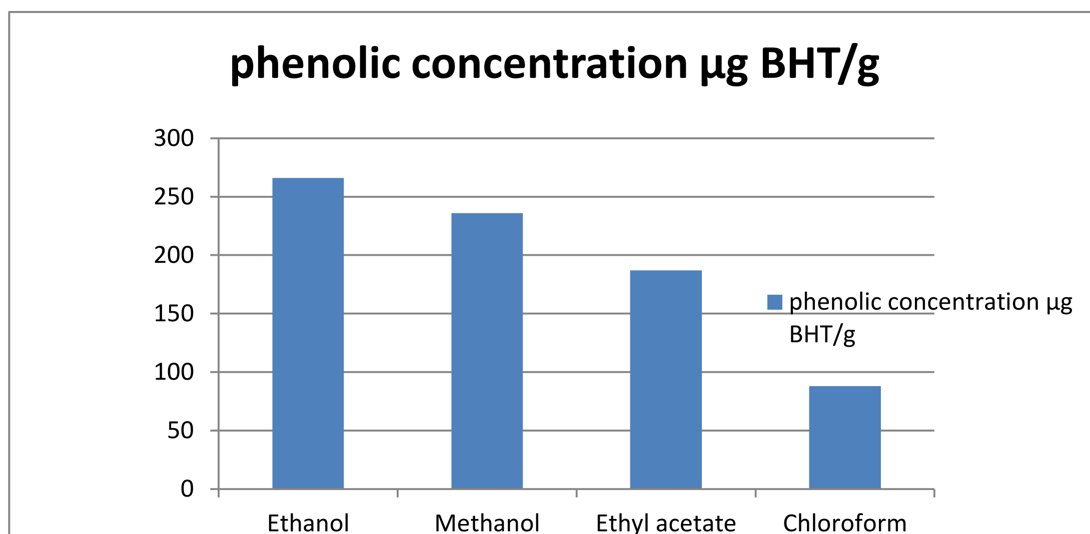
DPPH: Free radical scavenging capacities of the four different extracts of *P. ostreatus* was shown Graph 2. The strongest reducing power inhibition was identified as ethanol (86.33%) and methanol (75.21%) extract of *P. ostreatus*. Weakest inhibition was observed at ethyl acetate (71.26%) and chloroform (61.19%) extracts of *P. ostreatus*.

Table1

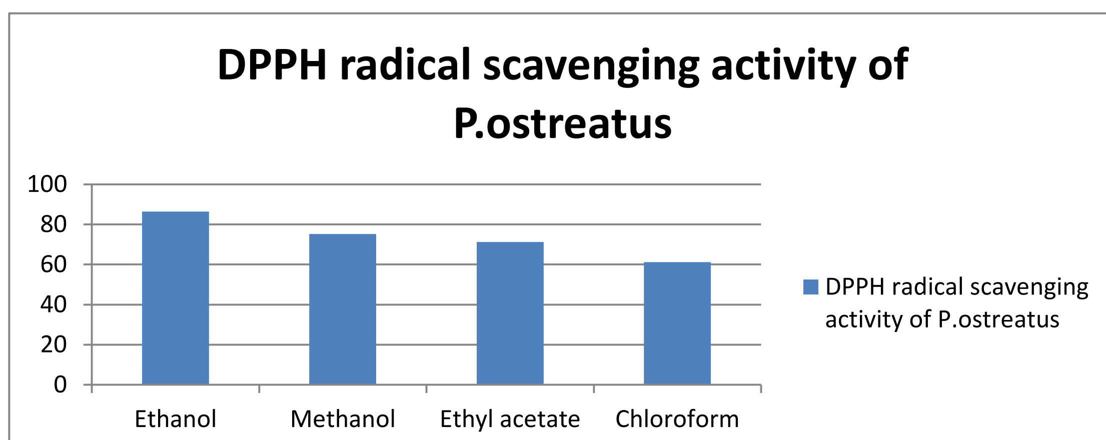
Different extract of *P.ostreatus*	**Phenolic concentration (µg BHT/g)**
Ethanol	248
Methanol	218
Ethyl acetate	187
Chloroform	88

Table 1

Showing phenolic concentration in different extract of P.ostreatus



Graph 1 Showing Phenolic concentration of *P.ostreatus* in different extract.



Graph 2 Showing DPPH radical scavenging activities of *P.ostreatus.*

Antioxidant activities which was observed that ethanolic extract of *P. ostreatus* shown in Graph 1 and 2. However, methanol also showed good antioxidant activity, next to ethanol while chloroform exhibited least antioxidant activity. This was agreed with the result of Vamanu et al reported that ethanol was the most appropriate solvent. If ethanol was used, *P. osteatus* strain had an antioxidant activity of 78.28%, it was higher than that of

methanolic extracts. Imran et al reported the total antioxidant activity of the methanol extracts were found to be 220 µg equivalent of BHT/gm of *P. florida* and 540 µg equivalent of BHT/gm of *P. eous* in phosphor molybdenum assay.

According to Gezer et al(2006) studied free radical scavenging activity. The 50% of inhibition value for Ramariaflava ethanol extract seem to be fairly significant when compared to commonly use synthetic antioxidants BHA and alpha tocophenols. Shahidi and Wanasundra(2007) indicated that the acetone extract possessed good activity, whereas the methanol and hot water extracts showed moderate and poor activity, respectively, at the concentration test. The variation may be attributed to difference in the concentrations of the antioxidant compounds / because of the solvent used for the extraction.

The phytochemicals or their secondary metabolites of mushroom present in the human diet possess a number of beneficial effects on human health such as anti-oxidant, anti-allergic, anti-viral, anti-diabetic, anti-inflammatory and anti-carcinogenic which was showed in the work of L.H.Yao et al in 2004. Similar results have also been observed especially in the *Pleurotus* spp collected. Anti-oxidant and anti-inflammatory activity might be responsible for *Gandoderma lucidium* as observed by R .Russel et al 2006. The relationship between the oxidative stress and inflammation has been investigated and reported in many edible mushrooms by Y. Ishitsku et al in 2007. With this background knowledge as supporting evidence in vitro pharmaceutical study was carried out.

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